Pathogenesis of the Henny Feathering Trait in the Sebright Bantam Chicken

INCREASED CONVERSION OF ANDROGEN TO ESTROGEN IN SKIN

FREDRICK W. GEORGE and JEAN D. WILSON, *Department of Internal Medicine and the Eugene McDermott Center for Growth and Development, University of Texas Southwestern Medical School, Dallas, Texas 75235*

ABSTRACT In female chickens of all breeds development of female feathering pattern is mediated by estrogens, whereas normal males and castrated chickens of both sexes develop male feathering. Male chickens carrying the henny feathering trait (such as the Sebright bantam and golden Campine) develop a female feathering pattern but otherwise virilize normally. To examine the possibility that the henny feathering trait is the result of increased conversion of androgen to estrogen in skin, estrogen formation from [1,2,6,7-^{3}H]testosterone was measured in tissue slices from control breeds and chickens with the henny feathering trait. Rates of estrogen formation were undetectable or low in all control tissues other than ovary, whereas rates were high in skin and skin appendages and detectable in many tissues from Sebright and Campine birds. The increased rate of estrogen formation in skin was demonstrable in Sebright chicks and in all areas of skin biopsied in the mature bird. Furthermore, plasma levels of 17β-estradiol were higher in Sebright and Campine than in control male cocks. Thus, increased formation of estrogen from androgen in the peripheral tissues probably explains the henny feathering trait.

INTRODUCTION

In most chickens a profound sexual dimorphism in plumage develops at the time of sexual maturation. In the male the feathers of the neck, cape, back, and shoulder are deeply fringed owing to the absence of barbules on the distal ends of the feather barbs, and the tail feathers are long and curved. In the female, except for the neck hackle and the fluffy abdominal feathers, all feathers have a solid vane with no fringing. This dimorphism is most apparent in the tail (Fig. 1). The development of the female feathering pattern is the result of a positive effect of ovarian estrogen whereas formation of male plumage is independent of the action of gonadal hormones (i.e., male plumage develops in males and females after bilateral castration [1].)

The Sebright bantam is a breed of chickens in which plumage is identical in the two sexes and in which the feathering pattern resembles that of the females of other breeds; Sebright bantam cocks are "henny feathered" (2). Because other male secondary sex characteristics (such as the comb) are normal in the male it was generally assumed that in some sense the feathers in the Sebright bantam must be resistant to male hormone; indeed, Fuller Albright and his colleagues (3), in deducing that the syndrome of pseudohypoparathyroidism is the result of a primary resistance to the action of parathyroid hormone, concluded that the disorder was analogous to the henny feathering trait and termed the disorder a "Sebright Bantam Syndrome" (3).[1] The concept of hormone resistance had important ramifications as it became recognized that resistance to the action of various hormones can cause endocrine disease (4).

The nature of the defect that underlies the henny feathering trait has never been elucidated. Castration of male Sebright and Campine chickens (another breed that carries the henny feathering trait) causes henny feathering to revert to normal male plumage (5, 6). Furthermore, transplantation of the testis from the Sebright cock to the caponized Leghorn chicken results in the development of a male feathering pattern

Received for publication 20 December 1979 and in revised form 19 March 1980.

[1] Following the initial usage of Morgan (5), Albright and his colleagues termed the breed Seabright rather than Sebright (3). This has resulted in a dichotomy over the years so that in the clinical literature the disorder is frequently designated the "Seabright bantam syndrome," whereas in the avian physiology literature the breed is termed correctly the Sebright bantam.

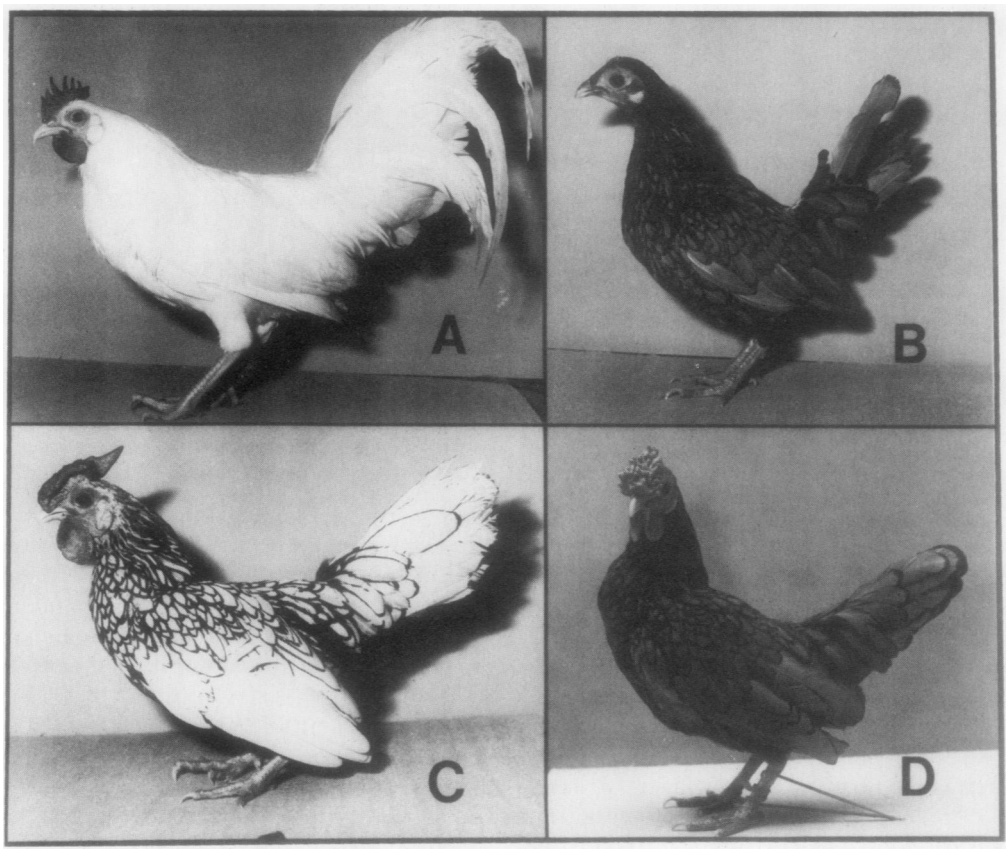

FIGURE 1 Feather pattern in normal chickens and chickens carrying the henny feathering trait. (A) white Leghorn bantam malè; (B) golden Sebright female; (C) silver Sebright male; (D) golden Sebright male.

and comb in the Leghorn, implying that the testis of the Sebright produces normal male hormones (7). Danforth and colleagues (8) then showed that when skin is transplanted from Sebright or Campine males to normal cocks, henny feathering persists in the transplanted skin whereas feathering is always of the donor type in transplants of skin from normal to henny males (9–13). Therefore, the defect must be present in the skin itself. This deduction is in keeping with the fact that the administration of testosterone to castrated Sebright cocks causes male feathering to revert to henny feathering, whereas similar treatment of castrated controls does not alter the normal male feathering pattern (14). In contrast, the administration of estrogen to castrated Sebright or normal cocks results in development of henny feathering in both breeds (15). Thus, the defect cannot be due to androgen resistance in the ordinary sense but must instead be due to the fact that testosterone acts aberrantly as an estrogen in skin of birds with the henny trait.

These various observations could all be explained if the henny trait caused an increase in the conversion of androgen to estrogen in the skin. In the human a significant amount of estrogen is formed from the peripheral aromatization of circulating androgens (16), a conversion that takes place in many tissues including the hair follicle (17) and fibroblasts cultured from skin (18). An increase in the rate of conversion of androgens to estrogens in the skin of birds carrying the henny trait could result in the local formation of estrogen and the development of feathering similar to that in normal females in which skin estrogen is derived principally from ovarian estrogen. To test this hypothesis we measured the conversion of androgen to estrogen in slices of various tissues from normal birds and from breeds carrying the henny feathering trait. We have also measured the androgen and estrogen levels in blood.

METHODS

Animals

Inbred birds were obtained from two sources: Mr. Bill Holland of Jerome, Idaho, and The Halback Poultry Farm, Waterford, Wisc. Breeds carrying the henny trait included the golden and silver Sebright bantams, both carrying the rose comb, and the golden Campine carrying a single comb. Control breeds included the silver spangled Hamburg bantam

carrying the rose comb, the white Leghorn bantam carrying a single comb, and the white Plymouth Rock bantam carrying a single comb.

Materials

Celite analytical filter was from Fisher Scientific Co., Pittsburgh, Pa., and silica gel G-HY thin-layer chromatography plates with plastic backs were from Brinkmann Instruments, Inc., Westburg, N. Y. [1,2,6,7-^{3}H]Testosterone (96 Ci/mmol), [1,2,6,7-^{3}H]androstenedione (90 Ci/mmol), 17β-[4-^{14}C]estradiol (50 mCi/mmol), 17β-[2,4,6,7,16,17-^{3}H]estradiol (150 Ci/mmol), and [4-^{14}C]estrone (50 mCi/mmol) from New England Nuclear, Boston, Mass. were purified by column chromatography on celite-ethylene glycol. Nonradioactive steroids were from Steraloids Inc., Pawling, N. Y. Anhydrous ethyl ether, petroleum ether (20–40C), methanol, toluene, acetic anhydride, analytical grade pyridine, nanograde isooctane (2,2,4-trimethyl pentane), ethyl acetate, and chloroform were from Mallinckrodt Inc., St. Louis, Mo. Dichloromethane was from Eastman Kodak Co., Rochester, N. Y. and ethylene glycol was from Matheson, Coleman, and Bell, Inc., Rutherford, N. J. Premixed scintillation cocktails were 3a20 from Research Products International Corp., Elk Grove Village, Ill., and Instagel from Packard Instrument Co., Downers Grove, Ill. Eagle's minimal essential medium was from Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y. Rabbit antiserum to 17β-estradiol was purchased from Micromedic Systems, Horsham, Pa.

Measurement of androgen metabolism in tissue slices

Incubation and extraction of tissues. Skin biopsies were obtained from living animals with the use of disposable 3 mm dermal punches. These biopsies and tissues removed at autopsy were cut with a razor blade into ~0.5 mm slices. The standard incubation mixture contained tissue slices (0.5–2 mg protein), 0.5 ml of Eagle's minimal essential medium and either 0.1 μM [1,2,6,7-^{3}H]testosterone or 0.1 μM [1,2,6,7-^{3}H]androstenedione. A blank containing no tissue was incubated with each series. The incubation tubes were gassed with 95% oxygen-5% carbon dioxide, capped, and incubated for 1 h with shaking at 37°C. After completion of the incubation 17β[4-^{14}C]estradiol and [4-^{14}C]estrone (~4,000 dpm each) were added to each sample to assess procedural losses. Solvolysis was then performed by a modification (18) of the method of Burstein and Lieberman (19). NaCl was added to each sample to give a final concentration of 3 M, and the solutions were acidified to pH 1.0 with 5 N H_2SO_4 and extracted twice with 5 ml ethyl acetate. The combined ethyl acetate extracts were incubated at 37°C for 24 h, neutralized with 1 ml 5% $NaHCO_3$, and backwashed three times with 1 ml water. A 0.2-ml aliquot was saved for subsequent thin-layer chromatography of C_{19} steroids. The remainder of the extracts were dried under nitrogen and redissolved in 5 ml 2% ethyl acetate in isooctane for celite column chromatography.

Separation of estradiol and estrone by celite column partition chromatography. Chromatography was performed on small columns of celite-ethylene glycol-H_2O as described (20). In brief, the samples containing ^{3}H-labeled metabolites and ^{14}C-labeled estrogen recovery standards were transferred to celite minicolumns, and the eluates were discarded. 3.5 ml isooctane were added, and the eluates were again discarded. Next, 3.5 ml 7.5% ethyl acetate in isooctane were added, and estrone was eluted from the column. Estradiol was then eluted with 5 ml 15% ethyl acetate in isooctane. The elution patterns of estrone and testosterone overlap in this system. Before the thin-layer chromatography procedures, 50 μg each of nonradioactive estrone and 17β-estradiol were added to the extracts containing the corresponding ^{14}C-labeled recovery standards.

Purification of estrone and estradiol by thin-layer chromatography. The solvent systems used for thin-layer chromatography are shown in Table I. Estradiol diacetate was formed directly and subjected to two thin-layer chromatographic procedures. Estrone was chromatographed once as the free steroid and once as estroneacetate. The steroids were detected on the plates by spraying with water. The areas containing the estrogen were scraped and transferred to disposable pipets filled with glass wool plugs, and the steroids were eluted with 5 ml ethyl acetate. Aliquots were transferred to glass scintillation vials and dried. 10 ml of 3a20 scintillation fluid were added, and the samples were assayed for ^{3}H and ^{14}C in a Packard 2650 Tri-Carb liquid scintillation spectrometer. The remainder of each sample was reserved for subsequent chromatography and acetylation or, in some instances, recrystallization. Steroids were acetylated at room temperature for 15–20 h with 0.1 ml pyridine and 0.1 ml acetic anhydride. The excess reagents were evaporated at 45°C under a stream of nitrogen, and the residues were redissolved in 0.05 ml dichloromethane for spotting on thin-layer plates. The formation of estrogen was calculated from the ^{3}H to ^{14}C ratio of each sample after the final chromatography procedure.

In some experiments samples of radioactive estrone acetate and estradiol diacetate that had been purified by thin-layer chromatography were mixed with 40 mg of the

TABLE I

Solvent Systems for Thin-layer Chromatography of Estrone and Estradiol Diacetate after Separation on Celite Minicolumns

Steroid	Chromatographic procedure	Solvent systems
Estrone	1	Dichloromethane-ethyl acetate-methanol (85:15:3)
Estradiol diacetate	1	Dichloromethane-ethyl ether (98:2)
Estrone acetate	2	
Estradiol diacetate	2	Dichloromethane-ethyl acetate (99:1)

TABLE II

^3H to ^{14}C Ratios of Estrone and Estradiol Recovered after Incubation of Skin Slices from Sebright Bantam Males with Radioactive Androstenedione and Testosterone

Substrate	Product	^3H:^{14}C Ratios								
		TLC			Crystallizations					
		Nonacetylated 1	Acetylated 1	Acetylated 2	ML1	ML2	ML3	ML4	ML5	Final crystal
[1,2,6,7-^3H]androstenedione	Estrone	10.9	7.5		8.7	9.1	8.9	9.1	8.7	9.6
	Estradiol		2.7	2.8	4.1	3.3	3.0	2.6	2.3	2.9
[1,2,6,7-^3H]testosterone	Estrone	4.2	3.6		3.9	3.9	3.8	3.9	4.1	3.9
	Estradiol		8.3	8.3	10.3	9.4	8.9	8.9	8.5	9.6

Slices of skin from golden Sebright bantam males were incubated for 1 h in 0.5 ml Eagle's minimal essential medium containing 0.1 μM [1,2,6,7-^3H]androstenedione or [1,2,6,7-^3H]testosterone. After the incubation ~4,000 dpm each of [^{14}C]17β-estradiol and [^{14}C]estrone were added. The tissues and media were extracted and subjected to celite columns and thin-layer chromatography (TLC) as described. After the final thin-layer procedure, 40 mg of the corresponding radioinert steroids were added, and five crystallizations were performed. The ^3H disintegrations per minute for the final crystals ranged from 580 to 2,000. ML, mother liquor.

corresponding authentic steroids and crystallized five times from a 1:1 mixture of ethyl-petroleum ether. There was an average change in the ^3H:^{14}C ratios of only ~10% after recrystallization of the estrogens as compared with the final thin-layer chromatography procedure (Table II).

Because both 17α- and 17β-estradiol can be formed in the chicken (21) and since our chromatography and recrystallization procedures do not separate the diacetates of the two epimers (22), an experiment was designed to determine the proportion of the two epimers synthesized in skin. The estradiol diacetate recovered after the second thin-layer chromatography procedure in two studies was dried under a stream of nitrogen and hydrolyzed with 2 ml 1.5% potassium hydroxide in methanol-water (9:1) for 48 h at room temperature. After addition of 100 μg each of 17β- and 17α-estradiol the samples were then extracted two times with 10 ml ethyl ether, and the ether layers were back washed with 5 ml water. The samples were dried under nitrogen, dissolved in ethyl acetate, and spotted for thin-layer chromatography. The plates were developed in chloroform-diethyl ether (60:40) which separates 17α- from 17β-estradiol (21). The marker steroids were visualized by spraying with water, and the spots were eluted and assayed for radioactivity as before. On average 41% of the ^3H was recovered in the area corresponding to 17α-estradiol and 59% in the area corresponding to 17β-estradiol. Thus, both epimers are formed in skin and skin appendages under the conditions of these experiments.

Chromatography of C19-steroids. For thin-layer chromatography of the C19-steroids an aliquot of the original ethyl acetate extracts containing ~50,000 dpm of ^3H was combined with a mixture containing 10 μg each of androstanedione (5α-androstane-3,17-dione), androstenedione, dihydrotestosterone (17β-hydroxy-5α-androstane-3-one), testosterone, and androstanediol (5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol). The samples were dried under air, dissolved in 20 μl dichloromethane, spotted on precoated sheets, and developed at room temperature in dichloromethane-ethyl acetate-methanol (85:15:3, vol:vol:vol). The steroids were visualized after spraying with anisaldehyde reagent (23). Each lane was cut into six bands, and the various sections were assayed for ^3H in a liquid scintillation spectrometer. 5α-reductase activity was estimated as the sum of all 5α-reduced steroids formed (androstanedione, dihydrotestosterone, and androstanediol).

Protein determination. After extraction of the steroids 3 ml 10% trichloroacetic acid was added to the aqueous layers containing the tissue residues. The tubes were placed in the cold for 1 h and centrifuged at 800 *g* for 20 min. The supernates were discarded, and the sediments were washed two times with ethanol, dried, and digested in 5.0 ml 1 N NaOH for 20 h at 37°C. Protein was determined by the method of Lowry et al. (24) using bovine serum albumin as the standard.

Measurement of serum steroids

Blood was allowed to clot at room temperature, and serum was obtained by centrifugation at 1,200 *g* for 10 min. Approximately 4,000 dpm each of ^3H-recovery tracers for androstenedione, testosterone, and 17β-estradiol were added to 1-ml aliquots of serum. Radioactive and nonradioactive steroids were then extracted two times with 5 ml ether. The ether extracts were taken to dryness and reconstituted in 0.5 ml 5% benzene in isooctane before chromatography on small celite columns.

Celite-ethylene glycol minicolumns were prepared and conditioned as before (25). The samples were transferred to the columns, and the eluates were discarded. The columns were then washed with 0.5 ml isooctane, and the eluates were discarded. Androstenedione was eluted with 5 ml isooctane. 5 ml 5% benzene in isooctane were washed through the columns and discarded. Testosterone was then eluted with 8 ml 20% benzene in isooctane. The columns were then washed with 5 ml 7.5% ethyl acetate in isooctane, and the eluates were discarded. Finally, 3 ml 30% ethyl acetate in isooctane were washed through the columns and discarded. Estradiol was eluted with an additional 5 ml 30% ethyl acetate in isooctane. The fractions corresponding to each steroid were dried under nitrogen and resuspended in methanol. A tenth of each sample was assayed for ^3H to assess recoveries, which were routinely 60–80%.

The radioimmunoassay procedures for androstenedione and testosterone (25) and for 17β-estradiol (26) have been described. Under the conditions of our assay 17α-estradiol cross reacts only 2.4% with the 17β-estradiol antibody.

RESULTS

When skin slices from male and female Sebright bantam chickens were incubated with radioactive androstenedione, approximately one third of the estrogen recovered was estradiol, and the remainder was estrone (Fig. 2). In contrast, when the substrate was radioactive testosterone, a third of the radioactivity was found in the form of estrone. Therefore, since both estrogens are formed from both substrates (indicating the presence of 17β-hydroxysteroid dehydrogenase in the skin) and since the presence of both 17α- and 17β-estradiol indicates the presence of 17α-hydroxysteroid dehydrogenase activity as well, rates of estrogen synthesis were subsequently expressed as the sum of estrone, 17α-estradiol, and 17β-estradiol formed. Estrogen formation in large amounts may result in an increased rate of estrogen sulfate formation, and, therefore, we also examined the effect of hydrolysis before purification of estrone and the estradiols. Solvolysis to hydrolyze estrogen sulfate resulted in an enhanced recovery of the total estradiol and estrone as compared with the recovery when this procedure was not done (free estrogens) (Fig. 2). Consequently, solvolysis was performed as a routine portion of the assay procedure. Estrogen formation in skin biopsies from normal control male and female chickens was undetectable using either androgen substrate whether or not solvolysis was performed (Fig. 2).

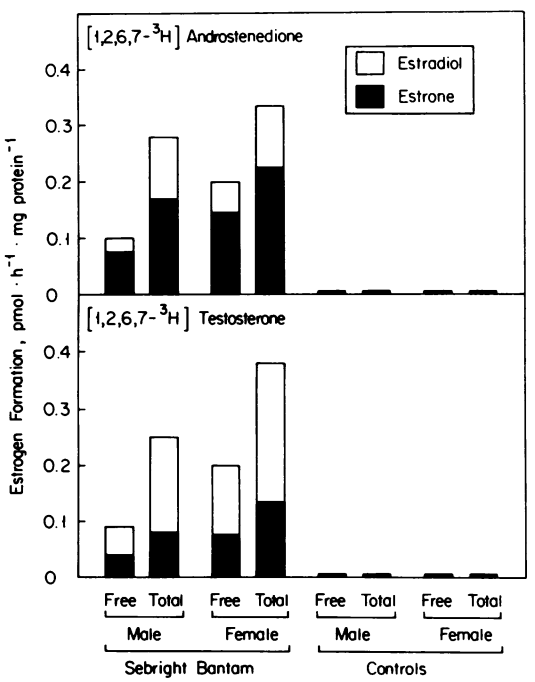

FIGURE 2 Free and total estrogen formation from [1,2,6,7-^{3}H]androstenedione or [1,2,6,7-^{3}H]testosterone in slices of tail skin from Sebright or control (silver spangled Hamburg) bantams. Two 3-mm skin biopsies from male or female chickens were incubated with either 0.1 μM [1,2,6,7-^{3}H]androstenedione or with 0.1 μM [1,2,6,7-^{3}H]testosterone. The free fraction represents the estrogens recovered in the absence of solvolysis, and the total fraction represents the estrogens recovered following solvolysis. Each bar represents the sum of estrone, 17α-estradiol, and 17β-estradiol.

Estrogen synthesis by Sebright skin slices increased as a function of substrate concentration up to about 0.1 μM testosterone, as a function of incubation time for ~1 h, and as a function of the amount of tissue in the incubation flask (Fig. 3). Standard incubation conditions were therefore adopted to include tissue slices (0.5–2 mg protein) incubated for 1 h at 37°C in 0.5 ml Eagle's minimal essential medium containing 0.1 μM [1,2,6,7-^{3}H]testosterone.

Estrogen formation was then estimated in skin biopsies from cocks that carry the henny feathering trait (silver and golden Sebright and golden Campine) and three control strains of bantam roosters (silver spangled Hamburg, white Plymouth Rock, and white Leghorn) (Fig. 4). In Sebright and Campine skin estrogen formation was easily demonstrable (0.15–0.60 pmol·h^{-1}·mg protein^{-1}) whereas estrogen formation was undetectable in skin from the three control strains. Since the ability of Sebright skin to form estrogens declines with age up to about 10 wk (Fig. 5) the difference in estrogen formation between Sebright and control birds is even more striking in skin biopsies from 2-wk-old chicks than from mature birds.

Enhanced estrogen formation is demonstrable in a variety of tissues of the Sebright in addition to skin (Fig. 6). In control birds estrogen formation is detectable only in ovary, whereas in the Sebright estrogen formation is active in skin, skin appendages (comb and wattle), and ovary and is demonstrable in many tissues including cerebrum, lung, spleen, adrenal, and crop. Similar results were found in tissues from two golden Campine males in that activity was high in skin from the chest, tail, and leg and in comb and wattle and demonstrable in lung (results not shown). Because the activity is measurable in an area of skin that does not contain feather follicles (leg skin) we conclude that the aromatase activity is located in the skin itself and not exclusively in feather follicles. We do not know whether this activity is in dermis or epidermis.

To ascertain whether the difference between control and Sebright bantams is specific for aromatase activity we estimated another pathway of steroid metabolism (5α-reduction) in the same experiments described in Fig. 6 (Table III). There was no significant difference in 5α-reductase activity in 21 tissues of control and Sebright chickens. There was also no apparent relation between 5α-reduction of androgens and the capacity to convert androgens to estrogens in various tissues of the Sebright. For example, 5α-reductase activity and aromatase activity are both high in some tissues (cere-

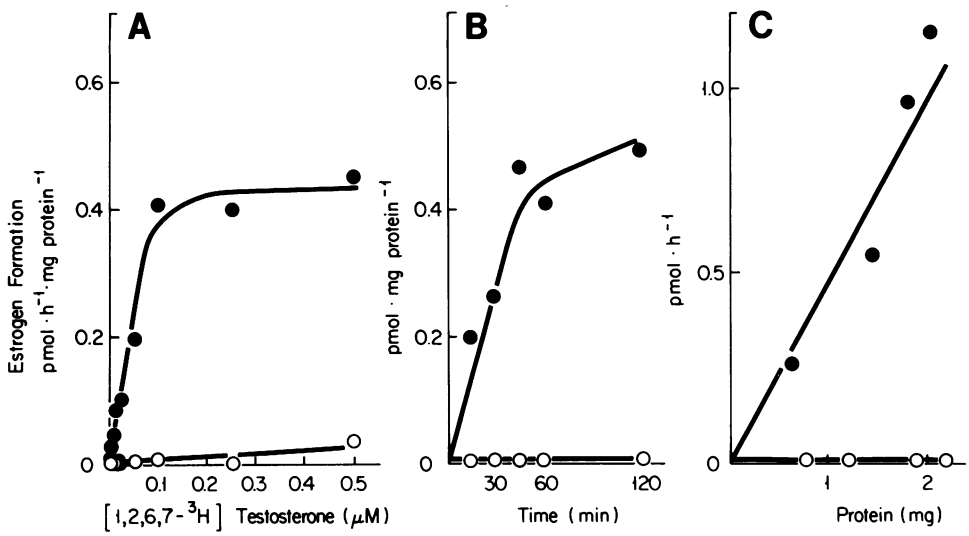

FIGURE 3 Estrogen formation in biopsies of tail skin from male Sebright (●) or control (○) (silver spangled Hamburg) bantams as a function of substrate concentration, time, and protein concentration. (A) Two 3-mm skin biopsies were incubated for 1 h at 37°C with concentrations of [1,2,6,7-^{3}H]testosterone that varied from 0.001–0.5 μM. (B) Estrogen formation was assessed in two 3-mm skin biopsies incubated at 37°C with 0.1 μM [1,2,6,7-^{3}H]testosterone for the times indicated. (C) Varying numbers of 3-mm skin biopsies (1–4) were incubated with 0.1 μM [1,2,6,7-^{3}H]testosterone for 1 h at 37°C. Estrogen formation (the sum of 17β-estradiol, 17α-estradiol, and estrone) was estimated after solvolysis as described in the text.

brum and comb), both low in some tissues (muscle and adipose tissues) and divergent in others (tail skin and liver).

The increased capacity of tissues from the Sebright and Campine birds to convert androgen to estrogen is reflected in the levels of serum 17β-estradiol (Table IV). The ratio of plasma testosterone to 17β-estradiol is more than 100 in the control males, 10 in the Sebright males, and 40 in the Campine males.

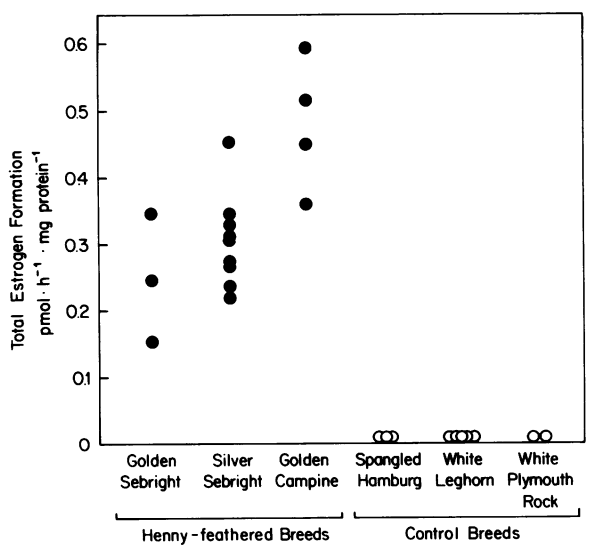

FIGURE 4 Comparison of estrogen formation in skin slices from 10 control, 12 Sebright and 4 Campine males. Total estrogen formation was assessed in two 3-mm biopsies of tail skin from males of the breeds indicated using the standard assay. The silver spangled Hamburg, white Plymouth Rock, and white Leghorn are bantam strains with normal feathering patterns.

DISCUSSION

Increased conversion of androgen to estrogen by peripheral tissues (including skin) can explain the

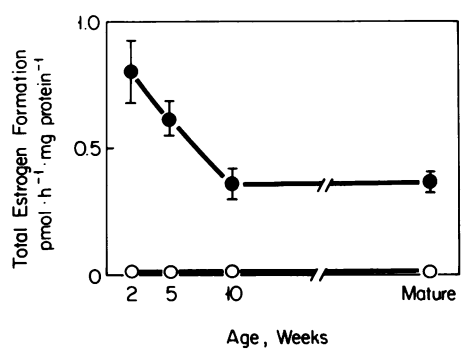

FIGURE 5 Estrogen formation in chicken skin in relation to age. Biopsies of tail skin were obtained from Sebright bantam and various control chicks at 2, 5, and 10 wk of age and from mature chickens throughout these experiments. Total estrogen formation was assessed as described in the text after incubating the skin biopsies with 0.1 μM [1,2,6,7-^{3}H]-testosterone. Each point represents the mean±SEM of four or more determinations.

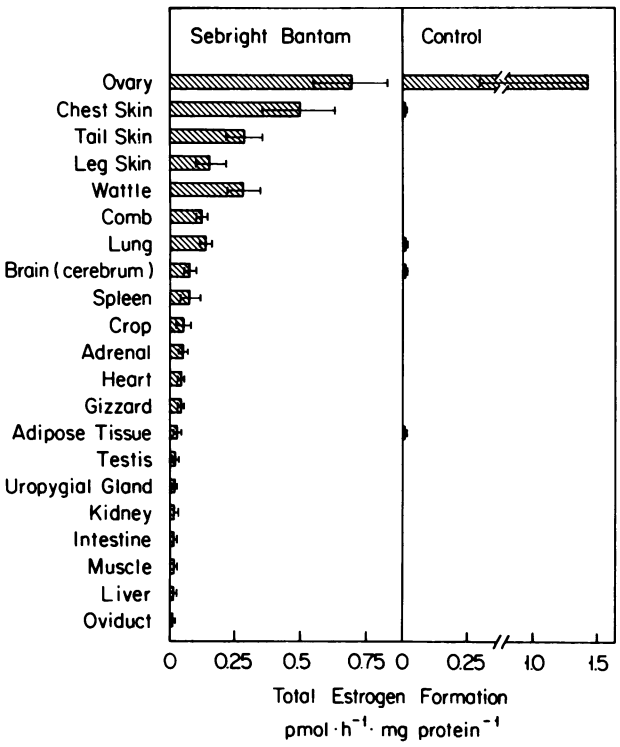

FIGURE 6 Estrogen formation in various tissues of the chicken. Slices (1–3 mg protein) from tissues of seven Sebright and six control (white Leghorn, white Plymouth Rock, and silver spangled Hamburg) bantams were incubated with 0.1 μM [1,2,6,7-^{3}H]testosterone at 37°C for 1 h as described in the text. The bars represent total estrogen recovery after solvolysis±SEM.

pathogenesis of the henny feathering trait in the Sebright bantam and Campine males. This explanation is in accord with the fact that estrogen is responsible for development of normal female feathering patterns in chickens and with the observation that the administration of testosterone (but not androgens such as androsterone that cannot be converted to estrogens) to castrated Sebright males results in development of the henny feathering pattern (27). Rigorous proof of this hypothesis will require demonstration that inhibition of aromatase activity results in formation of male feathering in affected chickens.

The nature of the genetic defect that gives rise to henny feathering is unclear. Morgan (28, 29) and Punnett and Bailey (30) interpreted the defect as the result of an autosomal dominant trait. The suggestion has also been made that the defect may have resulted from translocation of a gene from the Z chromosome to an autosome (31). However, two issues cloud the interpretation of the genetics. (*a*) Many affected male Sebright bantams are infertile (2), so that propagation of the defect is by trial and error. The reason for the high rate of infertility is unresolved. It is tempting to speculate that the elevated plasma estrogen may have a deleterious effect on testicular function. (*b*) Occasional normal feathered cocks appear in inbred colonies of Sebright bantams (31). The reason for this apparent reversion to the nor-

TABLE III
5α-Reductase Activity in Various Tissues of Sebright Bantam and Control Chickens

Tissue	5α-Reductase activity	
	Sebright bantam	Control
	pmol·h⁻¹·mg protein⁻¹	
Ovary	4.6±0.2	5.1±1.9
Chest skin	1.1±0.3	1.5±0.2
Tail skin	0.5±0.2	1.3±1.0
Leg skin	0.4±0.2	0.2±0.1
Wattle	3.5±0.3	3.8±0.9
Comb	6.4±1.3	5.4±1.9
Lung	0.9±0.1	2.7±0.1
Brain (cerebrum)	5.4±0.7	4.8±0.5
Spleen	3.4±0.8	2.7±1.1
Crop	3.0±0.4	3.1±0.9
Adrenal	1.3±0.7	1.1±1.1
Heart	0.1±0.06	0.1±0
Gizzard	0.4±0.2	0.5±0.4
Adipose tissue	0.3±0.3	0.7±0.3
Testis	0.5±0.2	0.6±0.4
Uropygial gland	0.4±0.3	0.7±0
Kidney	0.4±0.2	0.6±0.3
Intestine	1.0±0.2	1.3±0.3
Muscle	0.1±0.06	0.1±0.05
Liver	2.0±0.5	1.4±0.6
Oviduct	5.4±0.6	6.2±1.3

These data are derived from the same incubations described in Fig. 6. After incubating slices of the various tissues with 0.1 μM [1,2,6,7-^{3}H]testosterone as described in the text, an aliquot (2%) of the extracted incubation mixture was combined with 10 μg each of androstenedione, androstanedione, dihydrotestosterone, testosterone, and androstanediol, and chromatography for C19-steroids was performed as described in the text. Each value represents the mean of three to six observations±SEM.

TABLE IV
Steroid Levels in Chicken Serum

Chicken	No.	Steroid assayed		
		Testosterone	Androstenedione	17β-Estradiol
		ng/ml±SEM		
Control male	6	1.5±0.38	1.1±0.62	<0.01
Sebright male	9	1.9±0.30	1.1±0.30	0.18±0.08
Campine male	2	3.0	0.6	0.08

The control males include two white Leghorn, two white Plymouth Rock, and two silver spangled Hamburg. Mean values only are given for the Campine males.

mal is unclear. It is possible that males homozygous for the henny gene have low fertility rates and that the disorder is commonly perpetuated by breeding heterozygous males. If increased estrogen synthesis in the henny animals is a manifestation of the primary gene defect it may be possible to clarify the question of the genetics.

The defect that appears to underlie the henny feathering trait is different from the endocrinopathies that result from hormone resistance; namely the aberrant hormone action is due to abnormal hormone metabolism rather than to a defect in the machinery of hormone action that causes true hormone resistance (4). The defect is also distinct from other hereditary abnormalities of hormone metabolism such as steroid 5α-reductase deficiency and type 1 vitamin D-responsive rickets, both of which result from impaired conversion of circulating hormones to their active forms (4). The defect in the Sebright bantam is instead the result of excess formation of a metabolite (estrogen) that is normally formed in small amounts.

An interesting human counterpart to the henny feathering trait has been described in a boy with a feminizing syndrome due to increased peripheral conversion of androgen to estrogen (32). In that study, however, the tissue(s) responsible for the conversion was not identified, and it was not established whether the abnormality was the result of a mutant gene or was an acquired disorder. The defect in the Sebright bantam and Campine involves several tissues including skin, skin appendages, portions of the gastrointestinal tract, and spleen, although skin is of most quantitative importance. Furthermore, the defect is manifest at the earliest age examined (2 wk) and results in enhanced levels of circulating as well as tissue estrogen.

In normal men of all ages and in postmenopausal women, estrogen formation in peripheral tissues is the major pathway for biosynthesis of the hormone (16). Nevertheless, the factors that normally regulate aromatase activity in peripheral tissues are poorly understood. Identification of a mutation that results in a profound increase in this activity in peripheral tissues provides an opportunity to explore the mechanism of normal regulation. At present we do not know whether the increase in estrogen formation in the Sebright is the result of an increase in the activity of the aromatase enzyme(s), to the presence of an activator, or to the absence of an inhibitor to the reaction. It should be possible to utilize enzymatic, genetic, and tissue culture techniques to resolve these questions.

ACKNOWLEDGMENTS

We thank Jan Noble for technical assistance and Dr. William J. Wize for aid in the design of the experiments.

This work was supported by grant AM03892 from the National Institutes of Health.

REFERENCES

1. Domm, L. V. 1939. Modifications in sex and secondary sexual characters in birds. *In* Sex and Internal Secretions. E. Allen, C. H. Danforth, and E. A. Doisy, editors. The Williams and Wilkins Company, Baltimore. 227–327.
2. Tegetmeir, W. B. 1867. The Poultry Book. Routledge & Kegan Paul Ltd., London.
3. Albright, F., C. H. Burnett, P. H. Smith, and W. Parson. 1942. Pseudohypoparathyroidism—an example of Seabright-bantam syndrome. *Endocrinology.* **30:** 922–932.
4. Verhoeven, G. F. M., and J. D. Wilson. 1979. The syndromes of primary hormone resistance. *Metab. Clin. Exp.* **28:** 253–289.
5. Morgan, T. H. 1917. Demonstration of the effects of castration on Seabright cockerels. *Proc. Soc. Exp. Biol. Med.* **15:** 3–4.
6. Morgan, T. H. 1920. The endocrine secretion of hen-feathered fowls. *Endocrinology.* **4:** 381–385.
7. Roxas, H. A. 1926. Gonad cross-transplantation in Sebright and Leghorn fowls. *J. Exp. Zool.* **46:** 63–119.
8. Danforth, C. H. 1928. Cause of hen-feathering in Campine and bantam males. *Proc. Soc. Exp. Biol. Med.* **26:** 86–87.
9. Danforth, C. H. 1930. The nature of racial and sexual dimorphism in the plumage of Campines and Leghorns. *Biol. Gen.* **6:** 99–108.
10. Danforth, C. H. 1939. Relation of genic and endocrine factors in sex. *In* Sex and Internal Secretions. E. Allen, C. H. Danforth, and E. A. Doisy, editors. The Williams and Wilkins Company, Baltimore. 328–350.
11. Danforth, C. H. 1935. Testicular hormones and Sebright plumage. *Proc. Soc. Exp. Biol. Med.* **32:** 1474–1476.
12. Danforth, C. H. 1943. Gene H and testosterone in the fowl. *In* Essays in Biology. University of California Press, Berkeley. 159–167.
13. Danforth, C. H. 1944. Relation of the follicular hormone to feather form and pattern in the fowl. *Yale J. Biol. Med.* **17:** 13–18.
14. Gallagher, T. F., L. V. Domm, and F. C. Koch. 1933. The problem of hen-feathering in Sebright cocks. *J. Biol. Chem.* **100:** 47. (Abstr.)
15. Deanesly, R., and A. S. Parkes. 1937. Multiple activities of androgenic compounds. *Q. J. Exp. Physiol.* **26:** 393–402.
16. Siiteri, P. K., and P. C. MacDonald. 1973. Role of extraglandular estrogen in human endocrinology. *Handb. Physiol.* **2**(Sect. 7; Endocrinology): 615–629.
17. Schweikert, H. U., L. Milewich, and J. D. Wilson. 1975. Aromatization of androstenedione by isolated human hairs. *J. Clin. Endocrinol. Metab.* **40:** 413–417.
18. Schweikert, H. U., L. Milewich, and J. D. Wilson. 1976. Aromatization of androstenedione by cultured human fibroblasts. *J. Clin. Endocrinol. Metab.* **43:** 785–795.
19. Burstein, S., and S. Lieberman. 1958. Hydrolysis of ketosteroid hydrogen sulfates by solvolysis procedures. *J. Biol. Chem.* **233:** 331–335.
20. George, F. W., and J. D. Wilson. 1978. Conversion of androgen to estrogen by the human fetal ovary. *J. Clin. Endocrinol. Metab.* **47:** 550–555.
21. Common, R. H., R. S. Mathur, S. Mulay, and G. O. Henneberry. 1969. Distribution patterns of in vivo conversion products of injected estradiol-17β-4-^{14}C and estrone-4-^{14}C in the urines of the nonlaying and laying hen. *Can. J. Biochem.* **47:** 539–545.
22. Milewich, L., F. W. George, and J. D. Wilson. 1977. Estrogen formation by the ovary of the rabbit embryo. *Endocrinology.* **100:** 187–196.
23. Moore, R. J., and J. D. Wilson. 1975. Reduced nicotinamide adenine dinucleotide phosphate: Δ^4-3-ketosteroid 5α-oxidoreductase (rat ventral prostate). *Methods Enzymol.* **36:** 466–474.

24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein determination with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
25. George, F. W., E. R. Simpson, L. Milewich, and J. D. Wilson. 1979. Studies on the regulation of the onset of steroid hormone biosynthesis in fetal rabbit gonads. *Endocrinology.* **105:** 1100–1106.
26. George, F. W., L. Milewich, and J. D. Wilson. 1978. Oestrogen content of the embryonic rabbit ovary. *Nature (Lond.).* **274:** 172–173.
27. Callow, R. K., and A. S. Parkes. 1936. Production of oestrogenic substance by the bird testis. *J. Exp. Biol.* **13:** 7–10.
28. Morgan, T. H. 1919. The genetic and operative evidence relating to secondary sexual characters. *Carnegie Inst. Wash. Publ.* No. 285.
29. Morgan, T. H. 1920. The genetic factor for hen-feathering in the Sebright bantam. *Biol. Bull. (Woods Hole).* **39:** 257–259.
30. Punnett, R. C. and P. G. Bailey. 1921. Genetic studies in poultry III. Hen-feathered cocks. *J. Genet.* **11:** 37–62.
31. Punnett, R. C. 1937. Henny feathering in the fowl: a fresh interpretation. *J. Genet.* **35:** 129–140.
32. Hemsell, D. L., C. D. Edman, J. F. Marks, P. K. Siiteri, and P. C. MacDonald. 1977. Massive extraglandular aromatization of plasma androstenedione resulting in feminization of a prepubertal boy. *J. Clin. Invest.* **60:** 455–464.